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RESEARCH PAPER

The effect of citric acid supplementation on growth performance, phosphorus absorption and retention in rainbow trout (*Oncorhynchus mykiss*) fed a low-fishmeal diet

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Abstract

A.J. Hernández, S. Satoh, and V. Kiron. 2013. The effect of citric acid supplementation on growth performance, phosphorus absorption and retention in rainbow trout (*Oncorhynchus mykiss*) fed a low-fishmeal diet. Cien. Inv. Agr. 40(2): 397-406. Two feeding experiments were conducted to investigate the effect of a low-phosphorus (P) diet in combination with citric acid (CA) on rainbow trout growth performance and P utilization. In the first 12-week trial, duplicate groups of 30 fish (12.0±1.7 g) were fed one of five diets. The basal diet was formulated with low-P protein sources and 15% fishmeal (FM) without inorganic P supplementation. The test diets contained 0.5% monocalcium phosphate (MCP), 3% CA or both. A FM-based diet served as the positive control. The diet with MCP showed good growth and feed performance comparable to the control. Growth was not improved by the addition of 3% CA. In the second trial, duplicate groups of 20 fish (80.5±18.5 g) were offered one of four diets during a 12-week period. The basal diet from the first trial with MCP supplementation served as the positive control, and the same basal diet without MCP supplementation again served as the negative control. The test diets were supplemented only with CA at 1 and 2%. The diet with 1% CA had growth performance similar to the positive control. Thus, the addition of 1% CA to a low-FM diet containing no inorganic P liberated enough available P to produce juvenile fish performance similar to that achieved with a diet containing supplemental inorganic P.

Key words: Citric acid, growth performance, phosphorus utilization, rainbow trout.

Introduction

Phosphorus (P) is an essential dietary nutrient. It has more known functions for normal metabolic activity in the body of all vertebrate animals than any other mineral element. P is also a structural component of several biological molecules. Accordingly, any limitation of the P supply will be reflected in a generalized impairment of body function (Sugiura and Ferraris, 2004). However, the utilization of P from feeds that contain levels exceeding the minimum requirement and/or in an indigestible form is low (Sugiura and Hardy, 2000). Therefore, a considerable portion of the unretained dietary P is excreted. In intensive fish culture systems, it may represent

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an important source of aquatic pollution. Research studies on increasing dietary P utilization from plant origin feed ingredients have become imperative for aquaculture, primarily because of concerns about P pollution from effluents.

To moderate the environmental effects of P output from intensive fish culture operations and achieve optimum growth performance, it is important to maintain the available dietary P as low as possible according to the particular requirements of the cultured fish and to reduce excess P. The achievement of an adjusted P balance will depend on not only dietary supply but also the bioavailability of this element in different feedstuffs and the absorption rate (Liesegang *et al*., 2002). In general, fishmeal (FM) based diets provide a total dietary P that exceeds the minimum requirement for optimum growth. To formulate low-P diets, a large percentage of FM must be replaced with alternative protein sources that contain less P. Certain plant protein ingredients have been described as feasible alternatives for the partial replacement of fishmeal in diets for aquaculture. However, the availability of P varies widely among different plant feed ingredients, and the available P content of the diet may be insufficient. Reducing dietary P below the optimum level required will result in decreased growth and economic losses. For this reason, diets formulated with low-P plant ingredients need to be supplemented with an adequate source of soluble inorganic P to meet the requirement. Our previous experiments have indicated that an adequate combination of alternative plant low-P protein meals supplemented with 0.5% monocalcium phosphate (MCP) significantly reduces the P loading from rainbow trout diets without compromising growth (Satoh *et al*., 2003; Hernández *et al*., 2005). Feed quality improvement involving methods for increasing P utilization from plant raw materials is one of the principal strategies used to reduce the environmental impacts of aquaculture (Sugiura and Hardy, 2000; Gatlin *et al*., 2007). The enhanced availability of P, together with reduced dietary levels, will result in less P loading from fish feeds. Dietary acidification is not a new concept in animal nutrition; there has been considerable research into the effect of dietary acidification on mineral utilization in terrestrial animals (Boling *et al*., 2001; Liesegang *et al.,* 2002, Valencia and Chavez, 2002; Brenes *et al*., 2003). The supplementation of fishmeal (FM) based diets with citric acid (CA) can improve P and mineral utilization in rainbow trout (Sugiura and Hardy, 2000; Vielma *et al*., 1999). However, the renal excretion of P increases as a result of the improved absorption of dietary P produced by citric acid addition. To decrease nutrient excretion, supplementary sources of inorganic minerals in the diet must be reduced if the normally non-digestible components of the diet are released through the use of citric acid. If this effect can be reproduced in diets formulated with low-P plant alternative ingredients, the addition of citric acid would be a useful approach for reducing inorganic P supplementation and the total P in the excreta, thereby limiting the environmental impacts of fish culture effluents. More recent investigations have shown that organic acid supplementation in diets for marine and freshwater fish can increase the P bioavailability of plant protein ingredients, making it possible to reduce the fishmeal component and avoiding the use of supplemental phosphates (Sarker *et al*., 2007; Pandey and Satoh, 2008; Sarker *et al*., 2012; Khajepour and Hosseini, 2012).

The primary goal of the present study was to determine the inclusion level of CA that would reduce the requirement for supplemental inorganic P in a low-FM diet for rainbow trout without affecting growth and feed performance. Two feeding experiments were conducted to investigate the influence of a low-P diet formulated with alternative plant protein ingredients in combination with CA on rainbow trout growth performance and P utilization.

Materials and methods

Experimental diets

The basal diet (0P0CA, negative control) was formulated with 15% FM and alternative low-P

ingredients (defatted soybean meal, SBM; corn gluten meal, CGM; poultry feather meal, PFM; and blood meal, BM) (Table 1). Wheat flour and pregelatinized starch were used as carbohydrate sources and binders, and pollock liver oil and soybean oil were used as lipid sources. A chromium oxide mix (Cr_20_3:dextrin=1:1) was used as an inert unabsorbable marker to determine the absorption rate of dietary minerals in the diets. Dietary additions of MCP and/or CA were made at the expense of cellulose to the basal diet. The ingredients were thoroughly mixed mechanically (ACM-50 LAT, Aikohsha Mfg. Co. Ltd., Tokyo, Japan), and deionized water was added prior to pelletizing (AEZ12M, Shimadzu Ltd., Kyoto, Japan). The pellets were dried in a vacuum freeze-drier (REL-206, Kyowa Vacuum Tech. Co. Ltd., Tokyo, Japan) and stored at 5°C until use.

Table 1. Feed components of the basal diet.

Ingredients (%)	
Jack mackerel meal[1]	15
Defatted soybean meal[2]	24
Corn gluten meal[3]	15
Poultry feather meal[4]	4
Blood meal[5]	4
Wheat flour	11
Pollock liver oil[6]	5
Soybean oil[7]	5
Pregelatinized starch	5
P-free mineral premixture[8]	1
Vitamin premixture	3
Choline chloride (100%)	0.5
Vitamin E (50%)	0.1
Cr_2O_3 (50%)	1
Cellulose	6.4

[1]Chilean jack mackerel meal.
[2]Kanematsu Co. Ltd. Japan.
[3]Product of China sourced from Sakamoto Feed Co. Ltd. Japan.
[4]Heat dried powder, Japan Farm.
[5]Spray dried powder, AP301, American Protein Corporation, Iowa, USA.
[6]Rikken Vitamin Co., Ltd, Tokyo, Japan.
[7]Hayashi Chemicals Co., Ltd, Tokyo, Japan.
[8]Mineral premixture composition (%): sodium chloride, 5.0; magnesium sulfate, 74.5; iron (III) citrate n-hydrate, 12.5; trace element mix[c], 5.0; cellulose, 3.0.The trace element mix had the following components (%): zinc sulfate heptahydrate, 35.3; manganese sulfate, 16.2; copper (II) sulfate pentahydrate, 3.1; aluminum chloride hexahydrate, 1.0; cobalt chloride, 0.3; potassium iodate, 0.1; cellulose, 44.0.

In the first trial, the experimental diets were similar to the basal diet but were supplemented with 0.5% MCP, 3% CA or both in combination at the same levels as those used separately and were designated as 0.5P0CA, 0P3CA and 0.5P3CA, respectively. In addition, a FM-based diet (55% FM) without MCP or CA was used as the positive control. The pH of the diets ranged from 4.49 to 5.88, with the lowest values in the diets supplemented with CA. The total P content differed markedly among the diets, with values ranging between 7.1 and 15.7 mg g^{-1} (Table 2). The addition of 3% CA resulted in depressed growth performance, suggesting that this CA level might be too high for low-FM diets. Consequently, a second trial was conducted to further evaluate lower levels of CA in an attempt to determine the optimal concentration of CA in low-FM based diets without MCP supplementation. The same basal diet with 0.5% MCP supplementation was used as the positive control (0.5P0CA). The experimental diets supplemented only with CA at 1 or 2% were designated as 0P1CA and 0P2CA, respectively. The total dietary P ranged between 7.1 and 8.1 mg g^{-1} (Table 2).

Fish, feeding and experimental conditions

Eyed eggs of rainbow trout (*Oncorhynchus mykiss*) were obtained from Fuji Trout Farm of Shizuoka Prefecture Fisheries Experiment Station and hatched under laboratory conditions at the Laboratory of Fish Nutrition, Tokyo University of Marine Sciences and Technology. The fish were kept on a commercial rainbow trout diet until they grew to the designated sizes. Fish with an average body weight of 12.0±1.7 g and 80.5±18.5 g were randomly selected from stock and distributed into 60-L glass rectangular aquariums at a density of 30 and 20 fish per tank for each of the two different trials that were conducted consecutively. The experimental set-up in both trials was arranged in a completely randomized design with two replications per treatment, and the feeding was conducted for a 12-week period. The fish were

Table 2. Proximate composition of the experimental diets in the first and second trial.

	First Trial					Second Trial			
	FM	0P0CA	0.5P0CA	0P3CA	0.5P3CA	0.5P0CA	0P0CA	0P1CA	0P2CA
Nutrients (%)									
Crude protein	46.8	43.9	43.6	43.5	43.2	43.3	43.3	43.1	42.7
Crude lipid	15.2	14.2	14.0	14.1	14.1	14.4	14.4	14.4	14.4
Crude ash	9.6	5.7	6.0	5.6	5.9	6.0	6.0	6.0	6.0
Moisture	3.7	9.1	5.5	6.2	5.6	2.3	2.1	2.8	2.6
Energy (kcal/g)	5.2	5.3	5.4	5.3	5.2	5.4	5.4	5.4	5.3
Diet pH	5.88	5.87	5.78	4.57	4.49	5.87	5.97	5.27	4.77
Phosphorus (mg g^{-1})									
Total P	15.7	7.1	8.1	7.1	8.3	8.3	7.3	7.3	7.5
Estimated available P[1]	8.2	3.6	5.0	3.6	5.0	5.0	3.6	3.6	3.6

[1]Estimates calculated using values of P absorption reported for feed ingredients in rainbow trout (Satoh *et al*., 2003).

hand fed three times per day, six days a week to apparent satiation. The tanks were well aerated and had a continuous dechlorinated tap water supply in a semi-recirculating system with a rate of 0.6-1.0 L min^{-1}. The average temperatures for the first and second experiment were 15.0±1.7°C and 16.9±0.5°C, respectively.

Sample collection and chemical analyses

Individual fish weights were measured at the start of each trial and every four weeks thereafter until the end of the feeding experiment to determine growth changes and feed performance. The fish were starved for 24 h and anesthetized with ethylene glycol monophenyl ether (300 ppm) before weighing. From the initial stock of fish and from each tank at the end of the experimental period, five fish were randomly sampled and totally minced using an ultracentrifugal mill (ZM 100, Retsch GmbH & Co., Haan, Germany) fitted with a 0.5-mm screen. The homogenate was preserved at -20°C for whole-body chemical analyses. An additional five fish were collected at the end of the study for the analyses of vertebrae and plasma. Blood samples were drawn from the caudal blood vessels of each fish with heparinized syringes and immediately centrifuged using a high-speed refrigerated centrifuge (SRX-201, Tomy Seiko Co. Ltd., Tokyo, Japan). After centrifugation (3000 rpm, 10 min, 4°C), plasma was separated and stored at -18°C for further analysis. Plasma alkaline phosphatase activity (ALP) was determined using the automated analyzer for clinical chemistry SPOTCHEM EZ SP-4430 with the SPOTCHEM II reagent strip for ALP (Arkray Inc., Kyoto, Japan) according to the manufacturer's instructions. Chemical analyses of the diets, vertebrae and whole-body samples were performed in three replicates following standard procedures as described by Satoh *et al*. (2003). Samples for mineral analysis were digested with nitric acid using the MLS-1200 Mega Microwave Labstation System (Milestone, Sorisole, Italy). P was analyzed using visible light spectrophotometry (UV 265 FW, Shimadzu Corp., Kyoto, Japan) at 750 nm. Crude protein content was analyzed using the 2020 Digestor and Kjeltec Auto Sampler System 1035 (Tecator AB, Höganäs, Sweden). The values obtained were employed for calculating whole-body P and N retention. The total P and N loading (considering fecal and non-fecal excretion and expressed as kg/ton fish production) was based on P and N retention and calculated as the difference between the amount of nutrient consumed and the amount of nutrient retained. Feed performance was evaluated on the basis of weight gain, specific growth rate and feed gain ratio. The absorption study was performed using the Tokyo University of Fisheries (TUF) column system for feces col-

lection (Satoh *et al*., 2003). Feces were collected during the last week of the feeding experiments for three consecutive days, and the pooled samples from each tank were freeze-dried for later analyses.

Statistical analyses

For statistical comparisons, the effects of dietary treatments on the measured parameters were assessed with a one-way analysis of variance (ANOVA) using Systat 8.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences between individual treatments were detected with a Tukey test. Treatment effects or differences were considered to be significant at $P \leq 0.05$.

Results

Data on overall growth performance, feed utilization and the results of P and N utilization and estimated loading amounts in fish fed the experimental diets for both trials conducted in this study are summarized in Table 3. Mortality was not significant during the trials, and the fish remained apparently healthy in all treatments. The omission of supplemental inorganic P from the basal diet resulted in negative growth due to insufficient available P. The addition of 3% CA to the low-FM basal diet depressed the growth performance of rainbow trout compared with the other treatments. The combination of 3% CA and MCP in diet 0.5P3CA resulted in slightly improved growth performance comparable to diet 0P0CA, but the growth performance on this supplemented diet was still significantly lower than the performance obtained with the FM based diet and diet 0.5P0CA. In any case, growth was not improved by the addition of 3% CA to the basal diet during the feeding experiment. Therefore, a second trial was conducted to evaluate lower levels of CA to determine the most favorable concentration of CA in low-FM diets.

During the second trial, the growth and feed performance of fish fed on diet 0P1CA without MCP and supplemented with only 1% CA was

Table 3. Effect of citric acid dietary supplementation on growth performance, feed efficiency, phosphorus and nitrogen utilization of rainbow trout fed the experimental diets in the first and second feeding trial[1].

	Av. body weight (g)				Absorption (%)		Available P in diet	Retention (%)		Loading (kg t^{-1} production)[5]	
Diet	Initial	Final	SGR[2]	FGR[3]	P	N	(mg g^{-1})[4]	P	N	P	N
First trial											
FM	12.1	54.9±0.5 a	1.78±0.03 a	1.01±0.02 a	62.1 a	95.3 a	9.8	30.2 a	36.3 a	11.0 a	47.9 a
0P0CA	11.8	49.0±1.4 b	1.67±0.01 b	1.17±0.03 b	62.4 a	95.8 a	4.4	53.3 b	33.1 b	3.7 b	52.6 b
0.5P0CA	11.7	60.6±1.7 a	1.93±0.01 a	1.02±0.03 a	68.8 b	95.0 a	5.6	54.8 b	35.6 a	3.8 b	46.0 a
0P3CA	12.0	37.8±0.3 c	1.35±0.01 c	1.60±0.04 c	65.4 c	94.9 a	4.6	47.8 c	23.8 c	5.9 c	85.0 c
0.5P3CA	12.1	47.0±1.1 b	1.59±0.01 b	1.30±0.05 b	65.6 c	93.3 a	5.4	50.0 c	30.7 b	5.4 c	62.2d
Second trial											
0.5P0CA	80.5	156.1±2.2 a	1.18±0.05 a	1.13±0.01 a	55.2 a	94.8 a	4.6	51.1 a	36.4 a	4.6 a	49.9 a
0P0CA	80.4	145.9±4.2 b	1.06±0.06 b	1.33±0.07 b	44.7 b	93.8 a	3.3	38.9 b	30.7 b	6.3 b	64.1 b
0P1CA	80.8	155.7±3.8 a	1.17±0.03 a	1.20±0.05 a	57.6 a	95.1 a	4.2	54.5 a	35.0 a	4.1 a	51.2 a
0P2CA	80.4	131.8±0.9 c	0.88±0.01 c	1.60±0.07 c	58.1 a	93.8 a	4.4	42.4 b	27.9 b	6.1 b	79.0 c

[1]Values in the same column and group not sharing a common letter are significantly different ($P \leq 0.05$).
[2]Specific growth rate.
[3]Feed gain ratio.
[4]Based on P absorption.
[5]Based on retention.

Table 4. Whole-body P, vertebrae P and ALP in plasma of rainbow trout fed on experimental diets for both trials[1].

Diet	Whole-body P (mg g^{-1})	P in bone (mg g^{-1})	Plasma ALP (U L^{-1})
First trial			
FM	4.8	108.9±0.7a	252.0±16.9a
0P0CA	4.4	97.9±0.9b	310.0±12.7b
0.5P0CA	4.6	109.6±3.7a	296.0±5.6b
0P3CA	5.3	110.3±0.5a	205.4±7.7c
0.5P3CA	5.5	109.3±2.4a	225.0±12.7c
Second trial			
0.5P0CA	5.5	130.6±2.6a	238.7±0.5a
0P0CA	5.1	129.5±5.6a	251.7±6.1b
0P1CA	5.5	133.4±5.6a	140.6±9.0c
0P2CA	5.7	132.5±6.2a	136.1±12.0c

[1]Values in the same column and group not sharing a common superscript letter are significantly different ($P \leq 0.05$).

comparable to that of the control group throughout the 12 weeks of the feeding experiment. Again, the omission of supplemental inorganic P from the basal diet resulted in decreased growth, but the lowest average body weight at the end of the feeding period was observed in the group of fish fed on diet 0P2CA with 2% CA supplementation.

In the first experiment, dietary P was efficiently retained by the fish fed on diet 0.5P0CA, resulting in lower P loading values compared with the FM-based diet used as the control. No differences in the absorption of N were observed at the end of the second experiment. However, the values of N retention varied among the treatments, ranging from 27.9 to 36.4%. Higher amounts of N loading into the water were estimated for diets 0P0CA and 0P2CA. P absorption did not differ significantly among the diet supplemented with MCP and the diets supplemented with CA at 1 and 2%, whereas it was significantly lower for diet 0P0CA. The whole-body retention of P was also lowest in diet 0P0CA. The lowest value of P loading was observed in the group of fish fed on the diet supplemented with 1% CA. The loading of P by rainbow trout was reduced significantly by the addition of 1% CA compared with diets 0P0CA and 0P2CA but did not differ significantly from diet 0.5P0CA.

The values for P for the whole body and the vertebrae and the plasma ALP at the end of the feeding periods for both trials are shown in Table 4. In both trials, the fish fed diets that were not supplemented with MCP showed a lower P content in the vertebrae compared with the groups of fish in the other experimental treatments. We also observed a significant decrease in ALP activity on the diets supplemented with CA.

Discussion

As expected, the basal diet with no additional supplementation of P negatively affected the overall performance of the fish. These results were in agreement with our previous study, where 0.5% MCP addition to the same basal diet was sufficient for optimal growth in rainbow trout (Hernández *et al*., 2005), and with the results reported by Jahan *et al*. (2003), which indicated that more available P should be supplied in fish

diets with low available P content that do not meet the recommended P requirement for the fish. Dietary available P requirements for optimum growth, feed utilization and bone mineralization ranging from 4 to 8 g kg^{-1} have been reported for rainbow trout and other fish (Sugiura and Hardy, 2000). The estimated available P level in the basal diet employed in this study was approximately 3.6 mg g^{-1}, less than the reported minimum requirement for the normal growth of rainbow trout. However, many factors are known to influence the absorption of mineral elements by fish. The dietary availability of P may change markedly among feed ingredients; in addition, it may be influenced by feed processing, solubility in water, diet digestibility, particle size and interactions with other nutrients.

Previous studies on environmentally friendly feeds for aquaculture have emphasized that the retention efficiency of nutrients such as P and N is important for the evaluation of feed quality (Jahan *et al.,* 2003; Hernández *et al.,* 2005). The increase in fish growth performance and in apparent P absorption and retention for the fish fed diet 0P1CA, compared with the fish fed diet 0P0AC, confirmed the greater availability of P in low-FM diets supplemented with 1% CA. Our results also showed that the fish fed diet 0P1CA and 0.5P0CA supplemented with inorganic P to provide adequate concentrations of available P were similar in growth performance and in P and N utilization. Thus, the results of this study suggest that CA might have liberated enough available P from the ingredients used in the experimental diet to allow growth performance similar to that achieved with the same diet containing supplemental inorganic P.

The enhanced P utilization tendency in the fish fed the diet supplemented with CA in this study is consistent with the findings of Boling *et al*. (2001) from studies conducted with chicks fed corn-soybean meal diets. These studies found that CA improved the utilization of phytate-phosphorus in broiler chicks but did so only on a small scale in pigs. In contrast, other studies have failed to show such effects of CA. Boling *et al*. (2001) indicated that CA only had a positive effect on performance in diets with low level of available P and with a Ca: available P ratio similar to or greater than 4:1. Most likely, as demonstrated by these authors, CA addition to diets, with the liberation of additional P, would produce a greater Ca: available P ratio imbalance that could explain the negative effect on performance. This aspect of CA supplementation needs to be further studied in rainbow trout diets. Research studies on increasing dietary P utilization from feed ingredients have become imperative for aquaculture, primarily due to concerns regarding P pollution from effluents. Dietary acidification has been demonstrated to increase in significant proportion the availability of phosphorus from animal feeds, including fish feeds (Vielma *et al.,* 1999). Most of these trials on the acidification of fish feeds have been conducted during short periods of time using FM-based diets. Because there were no significant differences in feed consumption during the first four weeks of the feeding experiments, the reason for a negative response to concentrations of CA higher than 1% is unclear. Fauconneau (1988), working with partial replacement of protein by single amino acids or organic acids in rainbow trout diets, found that fish fed a CA-supplemented diet (approximately 12%) had very poor growth performance. Brenes *et al.* (2003) found that the inclusion of CA depressed the growth performance of broiler chicks but caused an increase in mineral utilization. Previous experiments conducted with *Seriola quinqueradiata* and *Pagrus major* have demonstrated that supplementation with organic acids can serve to reduce the fishmeal component in diets for these species and to avoid the use of inorganic phosphates by enhancing the bioavailability of P and other minerals present in dietary alternative protein ingredients (Sarker *et al*., 2007; Sarker *et al*., 2012). Our results are in agreement with the results of a study by Pandey and Satoh (2008) in which different organic acids were evaluated in low-fishmeal diets for rainbow trout. In particular, our study showed higher levels of phosphorus in carcass and bone in the fish fed diet 0P1CA. These

results suggest that dietary acidification with CA up to 1% supplementation is sufficient to enhance mineral utilization from ingredients used in the formulation of experimental low-fishmeal diets without affecting growth performance (Table 3).

The activity of metalloenzymes such as ALP is a potentially useful tool for diagnosing mineral status in rainbow trout (Skonberg *et al.*, 1997). In the present study, the fish fed diets supplemented with CA had low ALP activity in plasma (Table 4). However, ALP activities appear to be highly variable in fish, and the normal ALP activities reported for rainbow trout plasma range from 83 to 330 U L^{-1} (Skonberg *et al.,* 1997). Because plasma ALP activity is influenced by many factors, it is difficult to establish its relationship with dietary CA supplementation. ALP is a metalloenzyme known to have an important role in bone mineralization and to be affected by dietary Zn. Studies conducted with chicks had reported that a decrease in the dietary P level produces increased ALP activity (Brenes *et al.*, 2003; Ebrahimnezhad *et al.*, 2008; Nourmohammadi *et al.*, 2010). In these studies, dietary CA supplementation facilitated the liberation of phytate P and increased the plasma P concentration, decreasing ALP activity, as also observed in the present study (Table 4). As indicated by Brenes *et al.* (2003), this decrease might reflect a down regulation of ALP resulting from the increased availability of P.

Although a positive effect of adding 1% CA to low-FM diets was clearly demonstrated by our study, our experiments were not designed to study the mode of action of CA. The mechanism by which CA increases the availability of P in low-FM diets formulated with alternative protein ingredients is unknown. Several possible mechanisms can explain the ability of CA to promote dietary utilization and growth performance. Supplemental CA may function by enhancing phosphate hydrolysis in the stomach or may alter other physiological conditions that influence mineral utilization, such as the solubility of mineral complexes, chelation and competitive antagonism among elements in the gastrointestinal tract, thereby improving total P absorption (Vielma *et al.*, 1999). Another possible mode of action of CA is its effect on intestinal pH. However, CA would not be expected to have a large effect on intestinal pH because it is an organic acid that is metabolized rapidly (Boling *et al*, 2001). As mentioned above, it is difficult to assess the relative importance of the possible effects of low concentrations of CA on the improved responses of fish observed in the present study. Further research is needed to elucidate the mechanism of CA on dietary and/or ingredient P utilization by rainbow trout, contrasting *in vivo* and *in vitro* methodologies.

In conclusion, 1% CA supplementation in low-FM diets enhanced the utilization of P from dietary plant ingredients by an unknown mechanism and improved growth performance. In addition, it reduced the need for P supplementation of the feed, reduced P loading and, thus, reduced P pollution. Thus, CA provides another possible means for increasing P utilization and decreasing P excretion in rainbow trout fed diets formulated with alternative low-P ingredients. The addition of 1% CA to a low-FM diet containing no inorganic P liberated enough available P from the alternative ingredients to allow growth performance similar to that achieved with a diet containing supplemental inorganic P. Further research involving different organic acids combinations and the use of other mineral supplements is needed.

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Resumen

A.J. Hernández, S. Satoh y V. Kiron. 2013. Efecto de la suplementación de acido cítrico sobre el crecimiento y la absorción y retención de fósforo en trucha arcoíris *(Oncorhynchus mykiss)* alimentada con una dieta basada en un bajo contenido de harina de pescado y formulada con ingredientes proteicos vegetales alternativos. Cien. Inv. Agr. 40(2):397-406. Se llevaron a cabo dos ensayos de alimentación, con el fin de evaluar el efecto de dietas con bajo contenido de fósforo (P) e inclusión de ácido cítrico (CA) sobre el rendimiento del crecimiento en trucha arco iris y la utilización de P. En el primer ensayo, de 12 semanas de duración, grupos duplicados de 30 peces (12,0±1,7 g) se alimentaron con cinco dietas experimentales. La dieta basal fue formulada con fuentes proteicas bajas en P y 15% de harina de pescado (FM), sin suplementación de P inorgánico. Las dietas experimentales contenían 0,5% de fosfato mono cálcico (MCP), 3% de CA o ambos. La dieta control estuvo formulada con un alto contenido de FM. Los peces alimentados con la dieta con MCP mostraron un buen crecimiento y un rendimiento alimenticio comparable al grupo control. El crecimiento no fue mejorado por la adición de 3% de CA. En el segundo ensayo, grupos duplicados de 20 peces (80,5±18,5 g) fueron alimentados durante un período de 12 semanas. La dieta basal usada en la primera prueba con suplementación de MCP sirvió como control. Las dietas experimentales se complementaron sólo con CA a 1 y 2%. Los peces alimentados con la dieta con 1% CA tuvieron un crecimiento similar al control. Así, la adición de 1% de CA a una dieta baja en FM, que no contiene P inorgánico, liberó suficiente P disponible como para permitir un rendimiento similar al alcanzado con una dieta que contenía P inorgánico suplementario.

Palabras clave: Ácido cítrico, crecimiento, uso de fósforo, trucha arco iris.

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